

**FOOD PRODUCT COMPRISING CAROTENOIDS**Field of the invention

The present invention relates to an edible composition,  
5 preferably an edible emulsion, comprising a coloured compound,  
especially carotenoids and/or derivatives thereof.

Background of the invention

Carotenoids, in particular, beta-carotene, are well known and  
10 have been reported to protect cells from the reactive species  
(free radicals) that are suggested to play a role in immunity  
and cell-cell communication. It has also been reported to be an  
anti-ageing agent in human cells, and so slowing down the  
ageing process of the cells of humans.

15 Compounds belonging to the class of carotenoids are best known  
for their role as dietary antioxidants, although other  
potentially protective mechanisms for this group of compounds  
have been identified e.g. provitamin A activity. About 600  
carotenoids occur in nature of which the six major ones in the  
20 diet are  $\beta$ - carotene, lycopene, lutein,  $\beta$ -cryptoxanthin,  $\alpha$ -  
carotene and zeaxanthin. Although no thorough scientific base  
for claiming a direct relation between cause and effect of the  
carotenoid intake and disease incidence has been proven thus  
far, inverse associations between carotenoid intake or status  
25 and disease incidence have been found.

Supplementation of food products with one of the most commonly  
known carotenoids, beta-carotene, is well known.

Beta-carotene is a well known source as it is the pro-vitamin  
of vitamin A. It is furthermore used as a colourant in food  
30 products, for example to colour drinks, soups, tomato products  
and margarine type of products. In such products, as a general  
indication, amounts around 5 mg/kg are used. Such an amount  
changes the colour of a white product to be light yellow.

It will be appreciated that regular amounts may vary among different food product. Food products comprising around 5 mg/kg of carotenoids and no or few other coloured compounds are generally pale yellow of colour. The higher the carotenoid content, the more intense the colour is.

In particular in the Western world, consumers are reluctant to products of which the colour has changed from what they are used to. For example, margarine, butter, halvarin or salad oils, as well as mayonnaise, are considered unacceptable by consumers when the colour of such products is intense yellow, orange or even red. However, we have found that, at the same time, this type of products are excellent vehicles of the daily intake of amounts of carotenoids sufficient to obtain an elevated carotenoid status in human blood serum levels. Hence, a desire to add carotenoids to food products in amounts higher than needed for colouring, or in amounts above which any change in colour of the food product is still desirable or acceptable, is present.

In particular, there is a desire for food products that are fortified with coloured compounds like carotenoids and/or derivatives thereof, while the colour is comparable to the colour of regular products of the same composition, that are not fortified with these coloured compounds. Fortification is nowadays also desirable for food products which may lead to a reduction of the body carotenoids by their consumption, for example in food products which contain sucrose polyesters which are used as fat replacers, or contain materials like sterols and stanols, or esters thereof.

Fortification of food products with carotenoids or derivatives is known. For example EP-A-671,461 discloses the addition of significant amounts of carotenoids to oil. In WO-A-95/05747 the use of beta-carotene to reduce oxidation of fats and oils is

disclosed. Compositions fortified with carotenoids are furthermore disclosed in EP-A-354,600.

Several attempts were made in the past to apply enhanced amounts of carotenoids to food products without significantly  
5 changing the colour of the food products.

US 5,532,009 discloses food products fortified with a water soluble carotenoid/cyclodextrin complex. It is disclosed that the carotenoid is encapsulated in cyclodextrin. Although it is reported that colour intensity and degradation are minimized,  
10 we have found that dextrin encapsulation can not sufficiently serve to reduce the yellow and/or red colour intensity of products comprising increased levels of carotenoids.

#### Summary of the invention

15 It is an object of the invention to provide an edible composition which comprises a significant amount of carotenoids, i.e. an amount of carotenoids substantially higher than found these days in such products, but without a significant change of colour due to this increased amount of  
20 carotenoids, of these edible compositions. Another object of the invention is to provide an edible composition in which the carotenoids are bio-available. A further object of the invention is to provide an edible composition with carotenoids, having a high storage stability. One or more of the above  
25 objects are attained according to the invention.

Accordingly, the invention provides an edible composition comprising at least 15 mg/kg of one or more coloured carotenoids and having a yellowness factor as defined in this specification of less than 4000, and a yellowness index in the  
30 range of 1 to 90, the carotenoids being evenly distributed throughout the composition. Preferably the composition comprises at least 18 mg/kg of one or more coloured carotenoids.

Though higher levels than 150 mg/kg of carotenoids may be applied, it has been found that there is hardly any contribution to any of the benefits indicated of these amounts over such a level. Of course, highest amounts applicable depend  
5 on the amount of food taken on a daily basis. For example, a single portion of a margarine varies in different countries. But differences in daily portions is even larger between the different food products in which the carotenoids may be applied.

10 The carotenoids are evenly distributed throughout the composition. In case the carotenoids are present in a food product or emulsion in encapsulated form, evenly distributed means that the encapsulates are evenly distributed throughout the product or emulsion. This means that, if part of the  
15 composition is consumed, the level of carotenoids in the part (whichever one this is, and whichever the size of the part) will be as indicated. Preferably, the carotenoids being evenly distributed also means that food products, in which a part, that may be consumed by a human, is found that has a  
20 significantly increased concentration of carotenoids, compared to other parts of the same food product, are not incorporated in this invention. An advantage with the compositions of this invention is that if only a small portion of the edible composition is consumed, still an increased level of  
25 carotenoids is eaten. Hence, in all embodiments of this invention, the carotenoids are homogeneously distributed throughout the composition. In case encapsulates are applied according to this invention, the distribution of the encapsulates is such that the number of encapsulates is roughly  
30 the same throughout each part of the edible composition. The food products according to the invention can show the following benefit:

- the presence of an elevated amount of one or more compounds which have a strong colouring capacity, which compounds provide health benefits
- a colour comparable to that of similar products which do not contain such an elevated amount of one or more of these healthy compounds.

An edible composition has now been found that comprises increased levels of carotenoids.

In a preferred embodiment, products according to the present invention have a carotenoids level of at least 15 mg/kg, and preferably at least 18 mg/kg, and have a yellowness factor as defined in this specification, of less than 4000, and the yellowness index is in the range of 1 to 75. Preferably, compositions of the invention comprise at least 15 mg/kg carotenoids, more preferred at least 18 mg/kg carotenoids, and have a yellowness factor of less than 3333, more preferred of less than 2850, and a yellowness index which is in the range of 1 to 65.

In particular, preferred embodiments within the current invention compositions comprise at least 20 mg/kg, and have a yellowness factor of less than 2000, the yellowness index being in the range of 1 to 70.

Particular embodiments in which the edible composition or food products comprise more than 35 mg/kg carotenoids, and have a yellowness factor of less than 2000 and a yellowness index in the range of 1 to 70 can fulfill specific needs for food products which comprise significant amounts of carotenoids, without these food products having altered significantly compared to similar products not comprising these high amounts of carotenoids. This is even more the case for products comprising even more than 45 mg/kg with a yellowness factor of less than 2000 and a yellowness index of less than 90.

The invention further relates to particular food products fulfilling these criteria, methods for preparing such food products, and carotenoid comprising compositions for use in products so as to obtain products according to the  
5 specifications indicated above.

### Detailed description of the invention

Where weight percentages are indicated throughout this application, they are expressed as weight percentage (wt.%) on  
10 total product weight, unless indicated otherwise.

Edible compositions according to the invention comprise coloured compounds, said coloured compounds being carotenoids and/or derivatives thereof. Derivatives are for example chemically modified carotenoids that still show the desired  
15 anti-oxidant functionality.

The carotenoids according to the invention are those carotenoids that are coloured. Carotenoids that are essentially devoid of colour, for example phytoene and phytofluene, are not included in the carotenoids definition according to the  
20 invention. However such carotenoids, essentially devoid of colour, may be present in addition to the carotenoids according to the invention. Coloured carotenoid is herein defined as a carotenoid that shows absorbance, e.g. at least 10%, in its absorption or reflection spectrum in the range of 400-700 nm  
25 (visual range), preferably in the range 400-550 nm.

In this patent specification, where reference is made to carotenoids, both carotenoids and derivatives thereof are meant.

Preferred carotenoids are  $\beta$ - carotene, lycopene, lutein,  $\beta$ -  
30 cryptoxanthin,  $\alpha$ -carotene and zeaxanthin.

The most preferred carotenoids are  $\beta$ - carotene,  $\alpha$ -carotene, lutein and lycopene.

For all embodiments within the present invention, it is preferred that the edible composition is an edible emulsion, as with food emulsions, the problem of increasing colour upon increasing carotenoids level, and the undesirability thereof is  
5 largest. In many cases this type of product is in most cases desired as a white to pale yellowish product.

In a particular embodiment, the compositions comprise a carotenoid mixture of lutein, lycopene, and  $\beta$ - and  $\alpha$ -carotene. The use of palm oil carotene is preferred over synthetically  
10 obtained carotene. In a further preferred embodiment, the edible composition comprises between 15-40 mg/kg carotenoids, of which about  $2/3^{\text{rd}}$  of the carotenoid is lutein, about  $1/6^{\text{th}}$  is lycopene, with the remaining part being a mixture of  $\beta$ - and  $\alpha$ -carotene. The  $\beta$ - and  $\alpha$ -carotene mixture in this preferred  
15 embodiment is preferably obtained from a natural source, of which palm-oil is a suitable example.

Said coloured carotenoids compounds and/or derivatives maybe present in capsules, hence being present in encapsulated form. In another embodiment, also free coloured compounds like  
20 carotenoids may be present in addition to the encapsulated carotenoids. However it is preferred to keep the free coloured compound content as low as possible.

Several methods have been found to obtain the edible compositions of the current invention and food products  
25 consisting of, or comprising such edible compositions.

In one particular embodiment of the invention, the carotenoids are encapsulated. This embodiment is preferred over other embodiments.

Encapsulated form means said coloured compounds like  
30 carotenoids and/or derivatives thereof are at least partly, preferably fully retained in one or more capsules. The term encapsulated both refers to an embodiment wherein a thin

coating (capsule wall) is formed around particles or drops comprising the encapsulated material (for example carotenoids), and to an embodiment wherein the particles or drops comprising the encapsulated material are trapped within or throughout a  
5 matrix.

The term "encapsulates" is also used to indicate encapsulated carotenoids.

Several methods for encapsulating carotenoids have been found which may be used for the preparation of the compositions  
10 according to the invention. Suitable encapsulation methods include encapsulation with a protein layer, encapsulation through the preparation of complex coacervates, preparation of duplexemulsions, and the like. It has been found that with these encapsulation methods, a further improvement may be  
15 obtained by the addition of minor amounts of titanium dioxide in the coating or matrix material of the particle. The amount of titanium dioxide per particle depends on the size of the capsule and the amount of carotenoid which is to be enclosed in it. Suitably, 5-30 wt.% of titanium dioxide, and preferably 5-  
20 20 wt.% of titanium dioxide, based on the total weight of the particle is applied.

The encapsulated form may for example be embodied by encapsulation of the coloured compounds like carotenoids and/or derivatives thereof in crosslinked compounds, precipitated  
25 compounds or heat set compounds.

The products of the invention may be obtained by the addition of relatively large particles. Such particles are preferably, but not necessarily smaller than 300  $\mu\text{m}$  (average size of the particles present). Preferably the average particle size is  
30 less than 150  $\mu\text{m}$ , more preferably less than 50  $\mu\text{m}$ .

The size of the capsules would depend on the desired (visual) effect. For instance, to obtain products which appear not to contain a carotenoid fortification, i.e. which contain

increased amounts of carotenoids, without the consumer using the product clearly noticing a colour difference, suitably contain capsules which have a particle size of less than 50  $\mu\text{m}$ , preferably less than 30  $\mu\text{m}$ , most preferred from 1 to 20  $\mu\text{m}$ .

5 Preferably encapsulation of the coloured compounds like carotenoids and/or derivatives thereof is in compounds which are degradable in the human body. This results in a high bio-availability of the coloured compounds, e.g. carotenoids, present in the composition.

10 Carotenoids for use in edible compositions of the current invention may, for example, be encapsulated in proteins. Examples of suitable proteins are gelatin, caseins and whey proteins, although other edible proteins may also be suitable. Suitable encapsulation may for example be obtained by

15 crosslinked proteins on a fat globule surface. Optionally said crosslinking is obtained under application of an enzyme suitable as a catalyst for crosslinking of said protein or other compounds. Suitable enzymes are for example transglutaminases, peroxidases, laccases, tyrosinases or

20 combinations thereof. The selection of enzyme is believed to be related to the protein or compound that is used as a substrate for crosslinking as some of the mentioned enzymes are known to be substrate specific. A preferred combination is transglutaminase with protein, with the proviso that whey

25 protein if used is preferably in the denatured state.

In a preferred embodiment coloured compounds like carotenoids and/or derivatives thereof are encapsulated in a shell consisting essentially of heat set protein.

Preferably said encapsulates are protein coated fat globules, 30 whereby carotenoids are dissolved in said fat. The thus formed encapsulates may be made visible by microscopy and protein staining. The presence of protein coated fat globules may also be demonstrated by a method wherein the edible composition

comprising encapsulates is heated (for example to about 50 °C) and centrifuged. The protein content in the bottom layer is then determined. This is protein in solution. The protein in the creamed fat layer may also be determined. It will be

5 appreciated that protein capsules or shells obtained by way of a combination of any of the above methods, such as heating in combination with transglutaminase treatment, are also encompassed. It has been found that even stronger and more stable particles are obtained if more than one layer of protein  
10 is applied as coating of the carotene encapsulates.

Another suitable way to obtain encapsulation is encapsulation in edible polymers other than protein, whereby dextrans are excluded. Examples of such polymers are gum arabic, starch and calcium alginate polymers.

15 Encapsulates may be in the form of coacervates.

For encapsulation, in most embodiments it is preferred to dissolve and/or disperse the carotenoid or carotenoid mixture in an oil or wax, which oil or wax is then added to an aqueous solution of polymer or polymer mixture (for complex

20 coacervates) and treated so as to obtain the encapsulate.

Setting of the polymer mixture so as to obtain particles may be provided for by reduction of temperature, by crosslinking by the use of an enzyme or chemical compound, or, for example, by adjusting the pH, e.g., to levels below 6, preferably to a

25 level in the range between 4 and 5. The most suitable way will depend on the polymers used.

In view of their resistance to high shear conditions and storage stability, when applied in an emulsion, and capability of reduction of colour, preference is found for particles of

30 complex coacervates. For the preparation of such complex coacervates, reference is made to EP 790780, though for the current invention, also carotenoids are present as indicated above.

It has been found that the use of such particles, for example of gelatin and Arabic gum, crosslinked by use of glutaraldehyde, provide very good, stable particles. By the addition of titaniumdioxide in the coacervation process the titaniumdioxide is included in the particles. These particles of carotenoids in complex coacervates also including 5-30 wt.%, preferably 5-20 wt.%, based on the particle weight, of titaniumdioxide, are preferred. Such particles are capable of obtaining a significant reduction of carotenoids colour in food products, preferably edible emulsions, when compared to non-encapsulated carotenoids in such products. The particles so obtained may be applied at levels of carotenoids up to 40 times as much as present with products known in the art without a significant change of colour, compared to products comprising these amounts of carotenoids without any treatment.

Also encompassed within the current invention are encapsulates comprising carotenoids in which the carotenoids are encapsulated in complex coacervates of at least two polymers, and the encapsulates comprise 5-30 wt.% on particle weight, of titaniumdioxide.

In yet another embodiment of the invention, part or all of the carotenoids are present in free form, and the composition in which the carotenoids are applied is aerated. Coloured compounds like carotenoids in free form are coloured compounds that are not encapsulated or at least present in such a form that the colour of these compositions is clearly visible in a product comprising these coloured compounds. The reduction of colour intensity for such free carotenoids containing products is suitably achieved by aerating these products, for example by sparging the products, after their preparation, with a suitable, non-toxic gas. Examples of such gases include air, oxygen, and, preferably, nitrogen. Aeration should be at least 10%. It has been found that with aeration of 30 up to 50%

products may be obtained which are widely acceptable by the consumers. Such products differ in carotenoid level and aeration level, but are yet perceived as being products similar in colour to the products these consumers are used to. Aeration  
5 is a method in particular suitable for fat based food products of a consistency which allows maintenance of the air bubbles in the product. Good examples of such products include spreads such as butter, margarine and lower fat spreads, or mixtures thereof, cream cheeses or other soft cheeses, ice cream, and  
10 particular kinds of dressings and sauces, desserts, mousses, and the like.

Alternatively, very small water droplets may be added to the emulsion for colour reduction in a manner similar to that of aeration. This method is in particular suitable for water in  
15 oil emulsions. The very small water droplets preferably do not contain any further colouring ingredients.

In yet another embodiment, an edible composition is prepared in which part of the carotenoids are present in encapsulated form and part are present in free form. In this embodiment, the  
20 level of free carotenoids may be adjusted so as to set the desired colour of the food product. For example, a product may be prepared in which lutein encapsulates, lycopene encapsulates, and free carotene are used. In a particular embodiment, such a food product may comprise 5-15 mg/kg of  
25 lutein, 1-8 mg/kg of lycopene, present as separate encapsulates or as encapsulates containing both, and 3-8 mg/kg of a mixture of alpha and beta-carotene in free form.

It is furthermore desired that the free carotenoids and/or encapsulates are evenly distributed throughout the edible  
30 composition. Such may suitably be obtained by adequate mixing. Though homogenization may be applied, for some encapsulates, such as protein encapsulates, this method is less preferred as

high shear pressures could lead to breaking of the particles and hence colouring of the product if done at high pressures. Edible compositions according to the invention are characterised by a maximum Yellowness Index whilst comprising a significant amount of carotenoids. When applying these high amounts of carotenoid to food products of the same composition, but without any technical measure, the colour of such 'untreated' food products will be significantly higher than the yellowness index of the products of the current invention.

10 Without any treatment the Yellowness Index will be at least 10% higher.

In a specific and preferred embodiment, the edible compositions are used for the preparation of food products, and preferably of edible emulsions, and more preferred these are ready-to-eat

15 food products.

Food products encompassed in the invention are preferred to be common products which are often used by consumers on a daily basis in amounts different for each individual. The invention is especially applicable for fat based food products. Fat based

20 food products are food products (partially) based on fat and regarded by the consumer as "fatty type of products".

Preferably the fat based product has a fat content of at least 10 wt.%, more preferably at least 20 wt.%, more preferably at least 25 wt%.

25 Such fat based food products may be for example water free food products such as shortenings or cooking fats. Said fat based food products may also be water comprising food products, which may be for example water continuous or fat continuous or bicontinuous. Examples of water continuous products are cheese, dressings, including mayonnaise, ice cream, milk type drinks, (drink) yoghurt, toppings and fillings, low fat margarine like 40% or 20% fat spreads.

Examples of fat continuous products are margarine and butter, low fat margarines comprising for example 40 or 25% fat, and the like. Suitable fat based products are for example liquid margarines, which are pourable or pumpable products comprising generally from 60 to 95 wt.% fat. Bicontinuous oil and water comprising products are for example disclosed in EP-A-463,688. These preferred products according to the invention may be of any consistency, for example pourable such as coffee creamer, pumpable such as certain liquid margarines, squeezable such as food pastes, spreadable such as margarine or margarine like table spreads, spoonable such as dairy creams, or wrapper type of products such as shortenings.

Fat based food products of high carotenoids comprised without change of colour are preferably chosen from the group of shortenings, cooking fats, cheese, dressings, including mayonnaise, ice cream, milk type drinks, (drink) yoghurt, toppings and fillings, butter, margarine and low fat margarine. Products thereof used in the western world on a daily basis and so very suitable for daily intake of enhanced carotenoid levels are shortenings, cooking fats, butter, margarine and low fat margarine.

In such products, suitably 15-35 mg/kg, preferably 15-35 mg/kg carotenoids selected from the group consisting of lutein, lycopene, alpha and beta-carotene, or mixtures thereof, is present. In a specific embodiment, this type of product comprises 5-15 mg/kg of lutein, 1-8 mg/kg of lycopene, present as separate encapsulates or as encapsulates comprising at least both, and 3-8 mg/kg of a mixture of alpha and beta-carotene in free form.

In the current invention, the edible compositions, in particular the edible emulsions are not coloured strong yellow, pink, orange or red or a mixed-colour thereof despite the fact that they preferably comprise at least 15 mg/kg carotenoids,

which would under circumstances disclosed in the prior art, wherein carotenoids are present in free form without any further treatment lead to intensely red, orange or yellow products.

5 It will be appreciated that the Yellowness Index depends on the product concerned.

If spreadable food products like margarine, cheese or products like yoghurt are desired, said products preferably show a pale yellowish colour, evidenced by a Yellowness Index of from 40 to

10 70 preferably from 45 to 65.

If products like off-white dressings or mayonnaise are desired, said products are preferably characterised by a Yellowness Index of from 10 to 40.

The current invention is in particular very suitable for food  
15 products comprising fat or a fat replacer such as table spreads or margarine or butter or margarine- or butter-like products.

The terms fats and oils are used interchangeably in this document. Both by fat and oil is meant a triglyceride composition or a non-toxic material having properties

20 comparable with those of triglycerides, which material may be indigestible.

Fat may be of any source such as dairy fat, vegetable fat, fish oil or a combination thereof. Preferred fats have a high content of polyunsaturated fatty acid residues as these fats

25 are considered to have a beneficial effect on cholesterol levels in serum, in addition to the effect provided by sterols.

Fat may for example be selected from the group comprising sunflower oil, safflower oil, palm oil, palm kernel oil, illippe oil, linseed oil, rapeseed oil, linola oil, soy bean

30 oil, coconut oil or combinations thereof.

If a combination of fats is used, it is preferred that the resulting fat blend comprises at least 30 wt.% on total fat

blend, more preferred at least 45 wt.% of polyunsaturated fatty acids.

Preferred products comprise at least 5 wt.% fat, more preferred from 5 to 80 wt.% fat which is a common amount for products

5 such as margarine. According to a preferred embodiment the edible composition according to the invention comprises from 0.1 to 25 wt.% protein, preferably from 2 to 15 wt.%, more preferred from 2 to 5 wt.% protein.

In a recommendable embodiment of the invention, the food

10 products within the scope of the invention are storage stable. This implies that among others, products according to the invention preferably do not show increased colouring of the food products in the course of storage as a result of breaking of encapsulates.

15 For products according to the invention, the desired storage time depends on regular use. Such a food product needs to be storage stable for a time sufficient for transportation to for example a super market, and in addition thereto for a certain time after the product has been bought by a consumer.

20 Preferred products are storage stable for at least 4 weeks, preferably at least 10 weeks, more preferred around 4 to 9 months. This storage stability implies that preferred products show, throughout the product, when compared to a product obtained directly after production, an increase in Yellowness

25 Index, of less than 10% after 4 weeks, preferably at most 20% after 10 weeks, whereby the product obtained directly after production is the reference product.

In addition to the above-mentioned ingredients, food products according to the invention may optionally comprise further

30 ingredients suitable for use in these products. Examples of these materials are sugar or other sweetener materials, EDTA, spices, salt, bulking agents, egg yolk, emulsifiers,

stabilising agents, flavouring materials, colouring materials, acids, preserving agents, vegetable particles etc.

Food products according to the invention may also comprise protein. Such protein may for example serve to improve

5 structure or flavour characteristics. Said protein may be derived from any source, for example dairy protein.

Examples of suitable methods to produce fat continuous products like high fat margarine or water continuous products such as cheese are described at a later stage.

10 The edible compositions comprising encapsulated coloured compounds like carotenoids and/or derivatives thereof, may be added to a food product or to ingredients suitable to prepare a food product in any known way, or may be a food product in itself.

15 In order to obtain a food product of optimal quality, it is advisable that the carotenoid comprising edible composition is homogeneously mixed with edible material or at least with one ingredient of the edible material that forms a food product. A homogeneous distribution is believed to avoid that certain

20 parts of the food products contain high levels of carotenoid, whereas other parts of the food products contain low levels of carotenoids. This may result in a very variable daily intake of these healthy compounds.

Said coloured compound comprising edible composition may for  
25 example be added to the aqueous phase before mixing with the fatty phase (if present) or may be added to the final product under stirring to ensure homogeneous distribution of the carotenoid composition in the final product.

The edible composition may be included in a food product  
30 whereby said edible composition is for example in the form of an emulsion or in the form of a powder.

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Especially if a process is used wherein high shear regimes are applied, the coloured compound comprising edible composition is preferably added just before packaging.

The amount of encapsulated coloured compound comprising edible

5 composition that is added to the final food product is such that the amount of coloured compound on total weight is at least 15 mg/kg on total product. It is believed to be within the capabilities of the skilled person to calculate the amount of encapsulated composition to be added, starting from the  
10 initial amount of coloured compound like carotenoid that is present in said encapsulated composition.

According to another embodiment the obtained coloured compound comprising edible composition is used for the preparation of a fat continuous product such as a margarine or margarine like

15 products for example comprising from 5 to 80 wt.% fat. A preferred process to prepare such a margarine (like) product comprises the steps of emulsification of aqueous phase in a melted fatty phase, mixing the formed emulsion to ensure uniformity, cooling said emulsion in a shear unit, for example  
20 a tubular swept surface heat exchanger, to obtain crystallisation, working the resulting partially crystallised emulsion in for example a pin stirrer unit and packaging the resulting fat continuous product. The encapsulated coloured composition is preferably added just before packaging.

25 Optionally before packaging the emulsion is subjected to a resting treatment to increase the final product consistency. Said resting is for example carried out in a resting unit or a quiescent tube. Optionally the aqueous phase is pasteurised before mixing it with a fatty phase.

30 In the preparation of water free products like shortenings, the encapsulated coloured compound comprising edible composition is preferably added in dried, powdered form.

A preferred process to prepare a pumpable oleaginous food product comprises the steps of melting triglyceride fat in a shear mixer such as an A unit, cooling to below the alpha crystallisation temperature and subsequent, or prior to  
5 cooling, mixing the triglyceride fat with the above indicated aqueous phase. The resulting product is preferably stored at a temperature from 0 to 25 °C, and preferably between 0 and 15°C.

Water continuous cheese products like fresh cheese are for  
10 example obtained by a process comprising the steps of preparing a cream composition comprising an oil in water emulsion, subjecting this composition to acidification, concentrating the composition under whey removal, applying a homogenising treatment to the obtained concentrate to obtain the final  
15 product.

A water continuous spreadable product may for example be prepared by the method indicated above for fresh cheese, whereby optionally the whey removal and concentration step are left out.

20 The edible composition comprising encapsulated coloured compounds like carotenoids may be added at any stage during processing.

A bicontinuous product may suitably be prepared by a process comprising the steps of forming an oil and water comprising  
25 mixture, homogenising the oil and water mixture under such conditions that an oil in water dispersion is obtained with fat droplets having a preferred volume weighted mean diameter of less than 5 micrometer, cooling the dispersion under conditions such that coalescence of the oil droplets is induced. Cooling  
30 may be effected by passing the emulsion through for instance a cooling coil, or a scraped surface heat exchanger.

The encapsulated coloured compound comprising edible composition may be added at any stage during processing, preferably after homogenisation.

Dressings comprising for example from 0 to 60 wt.% fat may

- 5 suitably be prepared by preparation of an aqueous phase comprising for example flavour components, preservatives, thickeners and emulsifiers. A fatty phase comprising a fat such as sunflower oil, or a fat replacer, enriched with sterol compositions may be added to the aqueous phase under stirring.
- 10 The obtained mixture is preferably thoroughly mixed to obtain a pre-emulsion and then processed in a low shear device such as a colloid mill. A water continuous gel dispersion then results. The edible composition comprising encapsulated coloured compounds is preferably added after the colloid mill treatment.
- 15 The invention is now illustrated by the following examples.

### Examples

#### Determination of yellowness index and yellowness factor

- 20 The yellowness index (YI) is determined from samples at 15 °C, on a Tricolor LFM3 Colorimeter (Dr. Bruno Lange mbH, Dusseldorf, Germany). The Yellowness Index is calculated from the tristimulus colour (in X, Y and Z) of the sample, referred to the standard illuminant C (white plate) and the 2 °C
- 25 observer according to the formula:

$$YI = \frac{1.28 \times X - 1.06 \times Z}{Y} \times 100 \quad (1)$$

30

The sample is introduced into a powder cuvette, available from Dr. Bruno Lange GmbH, Dusseldorf, Germany. The powder cuvette is filled with the composition for three quarters, in such a

way that the bottom of the cuvette is completely and uniformly covered with the composition (without enclosed air bubbles).

The yellowness factor (YF) is the yellowness index (YI),  
5 divided by the carotenoids level expressed in g/kg.

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**Example A**

*Spreads containing 70 wt.% of fat were prepared as follows:*

In this example, amounts indicated are based on the final  
5 product.

A fat phase was prepared of ingredients:

63% sunflower oil

7% of an interesterified fat composition of 65 parts of a  
10 fractionated palm oil stearine and 35 parts of palm kernel  
oil

0.04% lecithin

0.08% emulsifier Hymono 8903<sup>tm</sup>

carotene (amount as indicated per example)

15 TiO<sub>2</sub> (amount as indicated per example)

Ingredients of the aqueous phase

1.75% NaCl

1% sweet whey

20 0.04% lactic acid

0.1% K-sorbate

water (balance amount)

pH was set around 5.0

25 The fat phase and aqueous phase were mixed to a pre-mix, and  
passed through an A-A-C processing line under the conditions as  
follows

The premix was heated to about 60 °C, and passed through the  
30 processing line for which the following conditions were  
applied:

A unit: 800 rpm, at 20 °C,

A unit: 800 rpm, at 8 °C

C unit: 200 rpm

The throughput was 4.5 kg/hr

5 *Preparation of carotenoid encapsulates of protein*

Carotenoid encapsulates were prepared as follows:

10 A 2.5% Na-caseinate solution was prepared by adding 37.5 g of Na-caseinate, 1461 g of demineralized water, and 1.5 g of potassium sorbate, and stirring for one hour.

15 To 1440 g of the so obtained solution, 160 g of a mixture of sunflower oil and  $\beta$ -carotene (4.8 g  $\beta$ -carotene, 155.2 g sunflower oil) was added and mixed, so that a turbid, dark red coloured mixture was obtained. The mixture was subsequently treated first by an Ultra Turrax<sup>tm</sup> and then by a high pressure homogenisator (pressure of 150 bar) to form an emulsion.

20 To the emulsion 0.75% D-gluconic acid lactone was added to lower the pH. The mixture was stirred in a vessel at 400 rpm for 45 minutes, and at 1800 rpm for another 45 minutes.

To the obtained mixture, 7.83 transglutaminase was added (1% enzyme), the mixture was stirred at 400 rpm for 17.5 hours. Subsequent centrifuging, filtering, washing, and repeating this, resulted in encapsulates containing 3010 mg/kg  $\beta$ -carotene. The encapsulates obtained are referred to as type "A".

*Preparation of carotenoid encapsulates of protein containing TiO<sub>2</sub>.*

30 The procedure of the preparation of carotenoid encapsulates of protein is repeated, the only alternative to the procedure being that after addition and mixing of the sunflower oil and carotenoids to the solution, 36 grams of TiO<sub>2</sub> were added. The

Ultra Turrax and homogenisator treatment were carried out as described above. As a result, encapsulates containing 8%  $\text{TiO}_2$  and 3214 mg/kg of  $\beta$ -carotene were obtained.

The encapsulates obtained are referred to as encapsulates type  
5 "B".

*Preparation of complex coacervates*

Carotenoid encapsulates were prepared as follows:

- 10 600 grams of a gelatin solution (concentration 2%, temperature about 60 °C) were added to 60 grams of a sunflower oil containing  $\beta$ -carotene (at 60 °C), and the mixture was stirred in an Ultra Turrax™. The amount of  $\beta$ -carotene was set so as to obtain a concentration of 1% in the sunflower oil. To the  
15 mixture, 600 grams of a 2 wt.% aqueous solution of Arabic Gum were added, the temperature was kept at 60 °C. After the addition of the Arabic Gum solution, an 28 grams of  $\text{TiO}_2$  were added under stirring. The pH was set at 4.3, using HCl (4 N), while the stirring was continued. The obtained mixture was  
20 allowed to cool to 20 °C under slow stirring, and subsequently cooled to about 4 °C.

The obtained encapsulates (coacervates) containing composition was filtered, and the encapsulates were obtained. To the encapsulates, water was added, the temperature of the

- 25 composition was raised to 50 °C, filtered again and the encapsulates where then washed with water.

To the coacervates, about 500 ml of water was added. Under slow stirring, 4.5 ml glutaraldehyde was added. Stirring was continued for about 19 hours.

- 30 By filtration and washing, 151 g of encapsulates was obtained. The  $\beta$ -carotene was present in an amount of about 1% of the total weight of the particles.  $\text{TiO}_2$  was present in an amount of about 30% based on total particle weight.

The encapsulates obtained are referred to as encapsulates type "C".

*Preparation of complex coacervates containing  $TiO_2$*

- 5 The procedure of preparation of encapsulated type "C" was repeated, with the proviso that the amount of  $TiO_2$  was 9.33 grams.

The encapsulates obtained are referred to as encapsulates type "D".

10

**Bio-degradability of complex coacervates "C" and "D"**

The bio-degradability of the complex coacervates "C" and "D" was measured using the enzyme mixture Pancreatin USP XXIII, in a test to simulate human intestinal conditions. The enzyme

- 15 mixture Pancreatin is comparable to the enzyme mixture in the human gastrointestinal tract, and is recommended by US Pharmacopeia (USP) for dissolution tests. Pancreatin USP XXIII was supplied by Sigma, Netherlands, product no. P8096, CAS no. 8049-47-6.

20

7 grams of encapsulates were mixed with 1 litre of a 0.79 wt.% solution of Pancreatin USP XXIII in water (at pH 7 and 37°C), and the breakdown of the the encapsulates was followed. The test resulted in a complete dissolution of the coacervates "C"

- 25 and "D" within 60 minutes.

The results of the above bio-degradability test indicate that the crosslinked coacervates according to the invention are most likely bio-degradable by humans and that therefore the

- 30 carotenoids in the crosslinked coacervates according to the invention have a high bio-availability.

**Comparative Examples I-V**

Spreads were prepared following the procedure as described above. Carotene was present in the in free form, i.e. not encapsulated, in amounts as indicated. Comparative example I  
5 corresponds to a commercial 70wt.% fat spread with beta-carotene colouring. No  $\text{TiO}_2$  was used. The carotenoids level on total spread was measured after the spread was prepared, and is indicated in Table I.

10

**TABLE I**

Ex.	$\beta$ -carotene (mg/kg)	$\text{TiO}_2$	Yellowness Index	Yellowness Factor (g/kg)
I	5	none	50	10000
II	22.4	none	92	4098
III	56.5	none	111	1965
IV	104.7	none	125	1194
V	153.5	none	134	873

**Example VI-VIII**

Spreads were prepared as described in the above procedure. This time, to the fat phase of the spreads, encapsulates of type "A"  
15 were added in an amount as indicated in Table II. No  $\text{TiO}_2$  was used.

**TABLE II**

Ex.	$\beta$ -carotene (mg/kg)	$\text{TiO}_2$	Yellowness Index	Yellowness Factor (g/kg)
VI	17.6	none	59	3352
VII	35.1	none	68	1937
VIII	103.1	none	88	853

It was observed that with these particles, it is preferred to add these after having passed the spread through the Votator processing line, under gentle stirring.

#### 5 Example IX to X

Spreads were prepared as described in the above procedure. This time, to the fat phase of the spreads, encapsulates of type "B" were added in an amount resulting in spreads containing an amount of the carotene as indicated in Table III.

TABLE III

Ex.	$\beta$ -carotene (mg/kg)	TiO <sub>2</sub> Present	Yellowness Index	Yellowness Factor (g/kg)
IX	25.4	yes	70	2756
X	62.8	Yes	90	1433

#### Example XI-XIII

Spreads were prepared as described in the above procedure. This time, to the fat phase of the spreads, encapsulates of type "C" were added in an amount in an amount resulting in spreads containing an amount of the carotene as indicated in Table IV.

TABLE IV

Ex.	$\beta$ -carotene (mg/kg)	TiO <sub>2</sub> Present	Yellowness Index	Yellowness Factor (g/kg)
XI	37.4	Yes	69.5	1858
XII	66	Yes	83	1257
XIII	48	Yes	87	1813

#### Example XIV-XVII

Spreads were prepared as described in the above procedure. This time, to the fat phase of the spreads, encapsulates of type "D"

were added in an amount resulting in spreads containing an amount of the carotene as indicated in Table V.

TABLE V

Ex.	$\beta$ -carotene (mg/kg)	TiO <sub>2</sub>	Yellowness Index	Yellowness Factor (g/kg)
XIV	15.0	Yes	31.34	2089
XV	44.3	Yes	43.96	998
XVI	95.1	Yes	50.13	527
XVII	158	Yes	60.61	384

5

**Example B**

10 *Spreads containing 40 wt.% of fat were prepared as follows:*

A fat blend was prepared of ingredients, amounts based on amount of fat blend:

73% bean oil

15 17% of an interesterified fat composition of hardened palmkernel oil

10% palm oil

This fat blend was used for the preparation of a fat phase as follows (amounts are based on total end product).

39.78 % of the above fat blend

0.05% lecithin

0.16% emulsifier (diglyceride of hardened palmoil)

0.012% of a 15 wt% beta-carotene dispersion in vegetable oil

25

Ingredients of the aqueous phase

1.1% gelatin

0.48% NaCl

0.27% acidic whey

0.12% K-sorbate

water (balance amount)

5 pH was set around 5.0 with citric acid

The fat phase and aqueous phase were mixed to a pre-mix, and passed through an A-A-A-C processing line under the conditions as follows.

10 The premix was heated to about 60 °C, and passed through the processing line for which the following conditions were applied:

A unit: 1000 rpm, at 20 °C,

A unit: 1000 rpm, at 14 °C

15 A unit: 1000 rpm, at 9 °C

C unit: 900 rpm

The throughput was 150 kg/hr

Nitrogen was inserted, after which gentle mixing took place.

20 Amount of Nitrogen is as indicated in the example.

The amount of carotene was measured, and the yellowness index was measured as indicated in the patent specification. The yellowness factor was determined by deviding the carotene level

25 with the outcome of the yellowness index measurement. The results are found in table VI.

TABLE VI

Ex.	$\beta$ -carotene (mg/kg)	N <sub>2</sub> Present %	Yellowness Index	Yellowness Factor (g/kg)
XVIII *	18	0	77.3	4294

XIX	18	17	68	3778
XX	18	23	60	3333
XXI	18	41	56	3111

\* Comparative example

This example shows that nitrogen is suitable for reducing colour of free carotenoids present in the food emulsion, at 5 nitrogen levels significantly higher than applied in the past.

#### **Determination of the spreadability of spreads**

The spreadability of the spreads was determined by spreading it on grease-free paper using a knife, and assessment whether the emulsion is stable after spreading. The result is compared with five standards on photograph. These standards vary from very stable emulsion (1) to complete separation of emulsion (5). All spreads according to examples I-XXI were very stable emulsions.